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Paralysis in nerve agent toxicosis in guinea pigs

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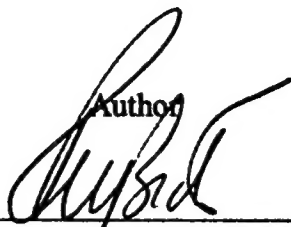
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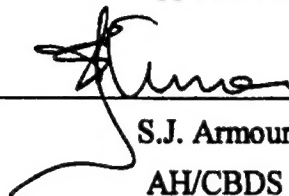
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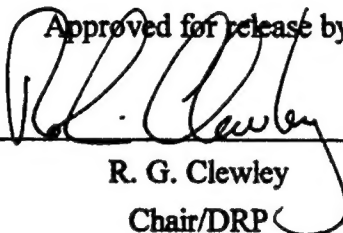
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DRDC Suffield Animal care statement

In conducting the research described in this report, the investigators adhered to the "Guide to the Care and Use of Experimental Animals, Vol. I, 2nd Ed." Published by the Canadian Council on Animal Care.

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Abstract

Since the discovery of the G-type nerve agents, there have been numerous studies of Central Nervous System (CNS) involvement in the etiology of organophosphate poisoning, notably associated with *status epilepticus* and/or "electrographic seizures". Brain damage from these effects has been demonstrated as "neuronal necrosis" primarily in the cortex, thalamus and hippocampus. However, despite recurring references to CNS damage, to the author's knowledge there have been no reports of long term paralysis manifested within 24 hr of nerve agent exposure. Occasional paralysis following an asymptomatic time interval of 7-14 days has been reported. This report is intended to summarize the immediate, nerve agent induced paralytic events recorded in guinea pigs (as incidental observations) during development of the Canadian Reactive Skin Decontaminant Lotion (RSDL®). Because the experiments were designed to assess the decontamination procedures, there were no apparent relationships between the amounts of agent applied and the sequelae recorded.

In the experiments described, massive cutaneous doses of agent were applied to large numbers of guinea pigs (GD to 1277; VX to 108) followed by decontamination with the RSDL® or predecessor lotions and solvents. The mortalities from GD (589) and VX (69) exhibited the classic clinical signs of nerve agent poisoning, namely excessive salivation (ptyalism), tremors, fasciculations, convulsions, apnea and flaccid paralysis before death. Of the 39 survivors of VX challenges, four exhibited the paralysis described following upon an insult which produced convulsions and/or flaccid paralysis. All of these were decontaminated with RSDL®. Of the survivors of GD challenges (688), many (348) exhibited some or all of these clinical signs. In addition, 84 animals recovering from the convulsions and/or flaccid paralysis showed apparently random, permanent paralyses of various parts of the body. Histological examinations of the brain and spinal cord of paralysed animals indicated encephalomalacia with occasional scattered foci of vacuolation in the brainstem and cerebellar white matter.

Of the 84 guinea pigs paralysed after GD challenge, one was not decontaminated, 25 were decontaminated with solvents only, either polyethylene glycol (PEG) or polyethylene glycol monomethylether (MPEG), 10 were decontaminated with potassium 2,3-butanedione monoximate (KBDO) - PEG and the remaining 48 were decontaminated with KBDO - MPEG (some variant of RSDL®). Thus, it would appear that the decontaminants had little effect on the resulting paralysis.

From these results, paralysis should be added to the list of concerns to be addressed when assessments are to be made of the medical requirements for treating, handling, support and long term care of casualties from nerve agent attacks.

Résumé

Depuis la découverte des agents neurotoxiques de type G, de nombreuses études ont examiné le rôle du système nerveux central (SNC) dans l'étiologie de l'empoisonnement par composé organophosphoré, associé notamment au *status epilepticus* et/ou aux « attaques électrographiques ». On a démontré que les lésions cérébrales qui en résultent sont des « nécroses neuronales » du cortex, du thalamus et de l'hippocampe. Bien que les lésions au SNC soient mentionnées de manière répétée, il n'a pas été observé, à la connaissance de l'auteur, de paralysie à long terme se manifestant dans les 24 heures qui suivent l'exposition à l'agent neurotoxique. On a observé des paralysies épisodiques selon des intervalles asymptomatiques de 7 à 14 jours. Ce rapport a pour objectif de résumer le déroulement de la paralysie immédiate causée par les agents neurotoxiques ayant été documentés chez les cobayes (comme observation auxiliaire) durant la mise au point de la lotion de décontamination de la peau (RSDL®). Les expériences n'ayant pas été conçues pour évaluer les procédures de décontamination, une relation apparente entre les quantités d'agents appliqués et les séquelles documentées n'a pas été établie.

Dans les expériences décrites, de massives doses cutanées d'agents ont été appliquées à un grand nombre de cobayes (1277 avec le GD et 108 avec le VX) et ont été suivies de décontamination avec la lotion RSDL® ou des lotions et des solvants prédécesseurs. La mortalité causée par le GD (589) et le VX (69) affichait les signes cliniques classiques d'empoisonnement par agent neurotoxique, à savoir, salivation excessive (ptyalisme), tremblements, fasciculations, convulsions, apnée et paralysie flasque précédant la mort. Parmi les 39 survivants à l'exposition au VX, quatre d'entre eux affichaient la paralysie décrite à la suite de l'agression, produisant des convulsions et /ou une paralysie flasque. Tous avaient été décontaminés avec la lotion RSDL®. Parmi les survivants à l'exposition au GD (688) beaucoup (348) affichaient quelques-uns ou la totalité de ces signes cliniques. De plus, 84 animaux qui se rétablissaient des convulsions et/ou de la paralysie flasque ont montré des signes apparemment aléatoires de paralysie permanente des différentes parties du corps. L'examen histologique du cerveau et de la moelle épinière des animaux paralysés a indiqué une ancéphalomalacie accompagnée quelques fois d'une dispersion des foyers de vacuolisation dans le tronc cérébral et la matière blanche cérébelleuse.

Parmi les 84 cobayes qui ont été paralysés après avoir été exposés au GD, un n'a pas été décontaminé, 25 ont été décontaminés avec seulement des solvants, soit du polyéthylèneglycol (PEG) ou du monométhyléther du polyéthylèneglycol (MPEG), 10 ont été décontaminés avec du potassium 2,3-butanedione monoxime (KBDO) - PEG et les 48 restants ont été décontaminés avec le KBDO - MPEG (une variante du RSDL®). Il apparaît donc que les décontaminants ont eu peu d'effet sur la paralysie qui a résulté.

Ces résultats indiquent que la paralysie devrait être ajoutée à la liste des inquiétudes à soulever quand seront faites les évaluations des exigences médicales pour le traitement, la manipulation, le soutien et les soins à long terme des victimes d'attaques d'agents neurotoxiques.

Executive summary

Background

Since the discovery of the G-type nerve agents, there have been numerous studies of Central Nervous System (CNS) involvement in the etiology of poisoning. These have been summarized in articles and textbooks. Some human cases have been described. However, despite recurring references to CNS damage, to the authors' knowledge, there have been no reports of long term paralysis manifested within 24 hr of nerve agent exposure. Occasional paralysis following an asymptomatic time interval of 7-14 days has been reported.

During the development of the Canadian Reactive Skin Decontaminant Lotion (RSDL[®]), which in the final form is a solution of potassium 2,3-butanedione monoxime (KBDO) in polyethylene glycol monomethylether (nominal molecular weight 550; MPEG) with 10% water, a large number of guinea pigs were exposed to massive cutaneous doses of agents GD and VX followed by decontamination either with the RSDL[®] or developmental lotions and solvents. Many of these animals exhibited the classic progression of clinical signs for nerve agent poisoning namely excessive salivation (ptyalism), tremors, fasciculations, convulsions, apnea and flaccid paralysis before death. Many died but many also "recovered", regaining muscle tone and control. A number of the recovering survivors showed permanent, rigid paralysis of various distal regions with varying severity. This note is intended to record and summarize these paralytic events.

Results

Of the 1277 exposed to GD, the 589 mortalities exhibited the classic progression of clinical signs for nerve agent poisoning defined above. Of the survivors (688), many (348) exhibited some or all of these clinical signs. A number of the survivors (84) showed paralysis of various distal regions with varying severity. Recovery in the case of paralysed subjects was defined as an animal physiologically functioning *i.e.* heart beating, breathing and with muscle tension. In the most severe cases, the animals were generally unresponsive and would neither eat nor drink. Respiratory function, heart beat and some reflexes could be established but mental activity appeared absent. In less affected animals, paralysis of all or some limbs was evident. If food and water were placed conveniently, many of these less affected survivors would eat, drink and continue, adjusting to their paralysis. Histological examinations of the brain and spinal cord of paralysed animals resulted in the diagnosis of encephalomalacia with focal necrotic lesions. In this study, there was no relationship to dose of nerve agent. The paralytic effects appeared to be random in occurrence and location.

Of the 108 animals exposed to VX, 69 died and showed the clinical signs described above. Twenty six survivors showed clinical signs up to and including the paralysis described above. Four of the 26 were paralysed in the hind quarters after recovering from convulsions and flaccid paralysis. All of the four were decontaminated with RSDL[®].

Discussion

Brain damage can result from severe exposure to GD, GB and other organophosphate agents. This study demonstrates and documents varying degrees of immediate and apparently permanent paralysis in survivors of GD poisoning. A similar paralysis was observed following VX poisoning. The paralysed animals had all suffered severe clinical signs of poisoning - at least convulsions and even flaccid paralysis. Brain damage, similar to that described in other studies, was present in those paralysed animals that were examined. Because the experiments described were not designed to demonstrate these effects or elicit causes or remediation, this report can only record the incidence of these effects. However, the experimental design does place emphasis upon the potential recovery from serious poisoning and may have contributed to the incidence of paralysis observed.

None of the animals received any form of prophylaxis or therapy other than the skin decontamination with the RSDL[®]. Applied topically, RSDL[®], itself, is non-toxic. Because there were no other therapeutic interventions, the effects of the current protective regimens proposed for nerve agent therapy cannot be assessed. However, the results of this study indicate that a paralytic outcome is possible when the poisoning is severe enough to result in convulsions and/or flaccid paralysis. Similar brain damage has been reported in other studies with GD, GB and other organophosphates in which decontaminants were not applied. It would appear logical to relate the paralysis directly to the brain damage. Therefore, a paralytic outcome should be considered when planning for the handling of casualties, whether military or civilian, as the load on medical services could be substantially increased.

Conclusions

A paralytic outcome is possible from severe nerve agent poisoning and should be considered in discussions of casualty handling and support.

Bide, R.W., Risk, D.J. and Schofield, L.N. (2002). Paralysis in nerve agent toxicosis in guinea pigs. (DRDC Suffield TR 2002-067). Defence R&D Canada – Suffield.

Sommaire

Contexte

Depuis la découverte des agents neurotoxiques de type G, de nombreuses études ont examiné le rôle du système nerveux central (SNC) dans l'étiologie de l'empoisonnement et elles ont été résumées dans des articles et des manuels. Quelques cas humains ont été décrits. Cependant, bien que les lésions au SNC soient mentionnées de manière répétée, il n'a pas été observé, à la connaissance de l'auteur, de paralysie à long terme se manifestant dans les 24 heures qui suivent l'exposition à l'agent neurotoxique. On a observé des paralysies épisodiques selon des intervalles asymptomatiques de 7 à 14 jours.

Durant la mise au point de la lotion de décontamination de la peau (RSDL[®]) qui est la forme finale du potassium 2,3-butanedione monoxime (KBDO) dans le monométhyléther du polyéthylèneglycol (poids moléculaire nominal de 550; MPEG) avec 10% d'eau, un grand nombre de cobayes a été exposé à des doses massives cutanées d'agent GD et VX suivies d'une décontamination soit avec la lotion RSDL[®] ou les lotions et les solvants en voie de mise au point. Beaucoup de ces animaux affichaient les signes cliniques classiques d'empoisonnement par agent neurotoxique, à savoir, salivation excessive (ptyalisme), tremblements, fasciculations, convulsions, apnée et paralysie flasque précédant la mort. Beaucoup d'entre eux sont morts mais beaucoup d'entre eux se sont aussi « rétablis », regagnant la maîtrise et le tonus musculaire. Un certain nombre de survivants en voie de rétablissement montraient des signes de paralysie rigide permanente de sévérité inégale dans des différentes régions distales. Cet article a pour objectif de documenter et résumer le déroulement de la paralysie.

Résultats

Parmi les 1277 qui ont été exposés au GD, les 589 qui sont morts ont affiché les signes cliniques de la progression classique de l'empoisonnement par agent neurotoxique tel que définis ci-dessus. Parmi les survivants (688), beaucoup (348) ont affiché quelques-uns ou la totalité de ces signes cliniques. Un certain nombre des survivants (84) indiquaient une paralysie de sévérité inégale des différentes régions distales. Le rétablissement des sujets a été défini comme un animal fonctionnant physiologiquement, qui par exemple respire, a le cœur qui bat et a une tension musculaire. Dans les cas les plus graves, les animaux étaient généralement insensibles et demeuraient sans manger ni boire. Les fonctions respiratoires, le battement du cœur et quelques réflexes ont pu être établis mais l'activité mentale apparaissait inexistante. Chez les animaux moins affectés, la paralysie de quelques-uns ou de la totalité des membres était évidente. Si la nourriture et l'eau étaient bien placées, beaucoup de ces survivants moins affectés se mettaient à manger et boire et continuaient à s'adapter à leur paralysie. Les examens histologiques du cerveau et de la moelle épinière des animaux paralysés ont diagnostiqué une ancéphalomachie avec lésions nécrotiques focales. Cette étude n'a pas établi la relation entre la dose et l'agent neurotoxique. Les effets paralytiques apparaissent survenir de manière aléatoire en ce qui concerne leur apparition et leur emplacement.

Parmi les 108 animaux exposés au VX, 69 sont morts en montrant les signes cliniques décrits ci-dessus. Vingt-six survivants ont montré la totalité des signes cliniques y compris la paralysie décrite ci-dessus. Quatre de ces 26 sont restés paralysés des cuisses pendant qu'ils se rétablissaient des convulsions et de la paralysie flasque. Tous les quatre ont été décontaminés avec la lotion RSDL[®].

Discussion

Les lésions cérébrales peuvent provenir d'une exposition grave au GD, GB et à d'autres agents de composés organophosphorés. Cette étude démontre et documente les degrés variés de la paralysie immédiate et apparemment permanente chez les survivants de l'empoisonnement au GD. Une paralysie similaire a été observée à la suite d'un empoisonnement au VX. Les animaux paralysés ont tous soufferts de signes cliniques graves d'empoisonnement - au moins de convulsions et même de paralysie flasque. Des lésions cérébrales, similaires à celles décrites dans les autres études, étaient présentes chez les animaux paralysés qui ont été examinés. Les expériences n'ayant pas été conçues pour démontrer ces effets ou déceler les causes ou la remédiation, ce rapport ne peut que documenter l'incidence de ces effets. Cependant, la méthodologie expérimentale qui met en évidence le potentiel de récupération après un empoisonnement, peut avoir contribué à l'incidence des paralysies qui y ont été observées.

Aucun animal n'a reçu d'autre forme de prophylaxie ou de thérapie que celle de la décontamination de la peau avec la lotion RSDL[®]. D'usage topique, la lotion RSDL[®] elle-même n'est pas toxique. Puisqu'il n'y a pas eu d'autres interventions thérapeutiques, les effets des schémas posologiques protecteurs proposés actuellement pour la thérapie des agents neurotoxiques n'ont pas pu être évalués. Les effets de cette étude indiquent cependant que la paralysie est possible si l'empoisonnement est assez grave et résulte en convulsions et/ou en paralysie flasque. Des lésions similaires ont été documentées dans d'autres études du GD, GB et autres composés organophosphorés dans lesquelles des décontaminants n'ont pas été appliqués. Il semble donc logique de relier les paralysies directement aux lésions cérébrales. Par conséquent, il faut prévoir un aboutissement à la paralysie dans la planification de la manipulation des victimes, qu'elles soient militaires ou civiles, ce qui risque d'accroître de manière considérable la charge de travail des services médicaux.

Conclusion

Il est possible qu'un empoisonnement grave aux agents neurotoxiques aboutisse à la paralysie et il faut prendre ceci en considération lors des discussions portant sur la manipulation et le soutien aux victimes.

Bide, R.W., Risk, D.J. and Schofield, L.N. (2002). Paralysis in nerve agent toxicosis in guinea pigs. (DRDC Suffield TR 2002-067). Defence R&D Canada – Suffield.

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Histology and diagnostics were done by Dr. J.P. Orr at the Department of Pathology, Western College of Veterinary Medicine, Saskatoon, Sask.

Introduction

Since the discovery of the G-type nerve agents, there have been numerous studies of Central Nervous System (CNS) involvement in the etiology of poisoning. These have been summarized in several articles and textbooks [1, 2, 3, 4, 5,]. Romano *et. al.* [3] provide a referenced review of previous texts. Some human cases have been described [5, 6]. However, despite recurring references to CNS damage from organophosphate poisoning [4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16], to the authors' knowledge there have been no reports of paralysis following close upon nerve agent exposure (within 24 hr).

The clinical signs of organophosphate poisoning vary in rate of onset, severity and duration according to the specific chemical involved, the route of administration and other factors [5, 8, 9]. Immediate effects such as excess salivation (ptyalism), tremors, convulsions and respiratory failure may be incapacitating or lethal and, in survivors, long term CNS effects can continue for extended periods up to the lifetime of the subjects. Delayed neurotoxicity is a common factor in chronic as well as acute organophosphate poisoning. The delayed effects begin to appear after extended periods in animals that appear unaffected immediately after exposure. The susceptibility differs between species [5]. Man is affected, along with some non-human primates, domestic chickens, dogs, cats and ruminants. Laboratory species, including the guinea pig, tend to be refractory, susceptible under specific conditions or react in an atypical manner. In these delayed responses, the descriptions are of a progressive distal degeneration of the axons resulting in incoordination and paralysis.

Brain damage has been reported in soman (GD) poisoning [8, 9, 10, 11, 12, 13, 14], in sarin (GB) poisoning [16] and in poisonings with other organophosphates [15]. Soman (GD) can cause axon degeneration in the rat brain [10, 11] which can be seen histologically using "specialized neuroanatomical research stains" and "...will escape early detection by the use of conventional methods". The effects of GD exposure apparently do not resemble those of experimental fetal hypoxia in monkeys or those resulting from delayed neurotoxicity in tri-ortho-cresylphosphate poisoning. The damage could be sparse to massive and has been found in animals that showed only mild clinical signs. The brain damage reported in GD poisoning has been associated with status epilepticus [12], epileptic type seizure activity [9, 13, 14] and/or "electrographic seizures" [13]. The brain damage has been reported as "neuronal necrosis" primarily in the cortex, thalamus and hippocampus [14]. Similar effects have been reported following GB poisoning [16]. These studies in rats included sampling of the cerebellum, brain stem and spinal cord where no lesions were reported. However, reference is made in one paper [14] to "occasional paralysis following an asymptomatic time interval of 7-14 days." Evidence of axon and terminal degeneration in many regions of the CNS has been reported in soman intoxicated rats [10], cats [8, 10] and monkeys [11]. Peripheral nerve damage has been reported in cats following organophosphate exposures [17]. Immediate distal paralyses are not mentioned in any of the above. As a complicating factor, one author refers to the problems encountered in studying the seizures because of the very steep dose-toxicity relationships involved [13].

During the development of the Canadian Reactive Skin Decontaminant Lotion (RSDL[®]), a large number of guinea pigs were exposed to massive, cutaneous doses of agents GD and VX

followed by decontamination with the RSDL[®] or predecessor lotions and solvents. Many of these animals exhibited the classic progression of clinical signs for nerve agent poisoning [1, 3], - namely ptialism, tremors, fasciculations, convulsions, apnea and flaccid paralysis before death. Many died but many also "recovered". A number of these "recovered" survivors showed varying degrees of paralysis in different areas of the body. This report is intended to summarize the paralytic events recorded (as incidental observations) during development of the RSDL[®].

Materials and methods

Chemicals

Pinacolyl methylphosphonofluoridate (GD, MW 182, CAS# 96-64-0) and O-ethyl S-(2-diisopropyl-aminoethyl) methylphosphorothiolate (VX, MW 268, CAS# 50782-69-9) were synthesized and purified by Mr. A. Hansen at DRDC Suffield. The LD₅₀ values used in these studies were 2.6 mg/kg and 80 µg/kg for the percutaneous application to depilated guinea pigs of GD and VX, respectively [18, 19, 20].

The RSDL[®] is a patented [21] preparation intended for skin decontamination of chemical warfare agents. The lotions used in these studies and the decontamination potency have been described in previous reports [22, 23, 24, 25, 26, 27, 28]. The RSDL[®] is a formulation of potassium 2,3-butanedione monoximate (KBDO) in a solvent - polyethylene glycol monomethylether (MPEG) of 550 nominal molecular weight.

Polyethyleneglycols (PEG) of various molecular weights, all of formulary grade, were used as the solvents in some of the decontamination studies.

All microliter doses of agent were dispensed using Western Model 800 positive displacement micro-volume dispensers (VWR Scientific, San Francisco, CA.).

Animals

Male albino guinea pigs, *Cavia porcellus*, virus free, Hartley strain [CRL(HA)BC], 250-350 g body weight were purchased from Charles River Canada, St. Constant, Que. Housing and husbandry have been described in detail [23]. All animals were acclimatized for at least 7 days in the Vivarium at DRDC Suffield before use. When used, most animals were between 450 and 750 g body weight.

Decontamination trial procedure

All trial procedures were similar to that used routinely in this laboratory in decontamination studies [23, 24, 25, 26, 27, 28]. Some early trials will have had minor deviations that should not have affected the results described in this report.

During chemical exposure and subsequent treatment, the animals were immobilized in a stainless steel restrainer that was developed at DRDC Suffield [18].

Sixteen to 48 hr before chemical exposure, the backs of subject guinea pigs were carefully shaved and depilated by application of Neet[®] depilatory cream (Rose scented Neet[®] was used exclusively because no skin emollients, oils or other modifying materials are added to this form of Neet[®]). After 20 - 30 min exposure to the depilatory, the animals were washed in running, tepid water and dried with paper towels. Vigorous rubbing was avoided. The animals were housed, individually, in stainless steel cages with mesh floors.

On the morning of the day of exposure, day 0, the animals were weighed, randomly distributed into experimental groups and placed in restrainers. At time 0, the prescribed dose of GD or VX was applied as a single drop on the middle of the back. After 55 sec, decontaminant¹ (0.7 mL) was applied to the target area from a polyethylene tuberculin syringe, taking care to cover the entire surface contaminated with agent. The decontaminant was gently rubbed on the skin for 20 sec with the syringe barrel to ensure full contact between the decontaminant, skin and remaining agent (all of this work was done before the introduction of the current applicator sponge [29]). After 1 hr, the decontaminant was wiped off using surgical gauze and, then, three one mL volumes of decontaminant were rapidly applied and removed. Each animal was washed, first with tepid tap water, then with 0.5% (v:v) Savlon[®] disinfectant solution² and then washed again with tap water. After drying with paper towels, the animals were returned to their individual cages. The animals were watched for clinical signs of poisoning throughout exposure, decontamination and the immediate post-treatment periods. They were examined daily for 3 days thereafter, and were weighed on days 1, 2, 3 and on the two following Monday mornings. The survivors were sacrificed on day 24.

Histopathology

Complete brains and spinal cords and tissue samples of heart, spleen, liver, kidney and muscle and were taken from some paralysed animals, fixed in 10% formalin and shipped to the Department of Pathology, Western College of Veterinary Medicine, Saskatoon, Saskatchewan for processing and examination. The samples were imbedded, stained according to standard procedures with hematoxylin and eosin and sectioned prior to examination by light microscopy.

¹ The usual decontaminant was RSDL[®]. In some experiments other materials were used (see Table 1).

² The procedure was originally developed for studies of HD decontamination. The Savlon[®] disinfectant was applied to reduce infection of the HD burns. The procedure continued as a routine process.

Results

Effects of GD

The doses of GD applied to the guinea pigs (Table 1) were intended to produce a 50 per cent lethal effect following application of the decontaminant of the day. As the potency of the decontaminant increased, the doses increased accordingly. Consequently, the doses varied from 0.82 to 73.6 μ l GD or 0.5 to 24 LD₅₀. The average dose was 15.4 μ L or 8.4 LD₅₀ of GD. As a result, there were no apparent dose-response relationships among the various groups of guinea pigs - dead, alive, affected and unaffected. The doses and ranges (Table 1) were statistically similar (*F* - test: *P* < 0.05).

Of the 1277 guinea pigs exposed to GD in these studies (Table 1), 1265 were decontaminated after about one min exposure (55 to 60 sec according to the immediate experimental protocol applied) and then again after one hr with the same decontaminant. The 14 others were part of an experiment to check the percutaneous toxicity of GD and were decontaminated with soap and water after one hr. Forty-six per cent of the guinea pigs exposed to GD succumbed to the nerve agent exposure. All of the mortalities were the result of nerve agent poisoning and showed the classic signs of the toxicosis. The affected animals expressed, progressively, the usual sequence of clinical signs associated with organophosphate poisoning, namely, ptyalism, tremors, convulsions, prostration (flaccid paralysis), apnea and death. Thirteen per cent also responded with opaque white tears in the later stages of the toxicosis. All of those affected had a clinical sign recorded before one hr and, generally, the sequence was completed or in remission within three hr.

For the animals listed as unaffected in Table 1, no clinical signs were observed.

Of the survivors, 51 per cent (348 animals) showed clinical signs of nerve agent toxicity with varying severity and either recovered or were "recovering" when terminated for humane reasons (*vide infra*). The paralysed animals were all placed in this survivor group. The incidence of paralytic effects from GD (Table 1) was 24 per cent in the animals that survived the poisoning and showed clinical signs. Of the total number of affected animals - including the mortalities - the incidence was 8.9 per cent.

Five decontaminants were applied (Table 1). Paralysis was observed with all of them. Two polyglycol solvents were used with and without KBDO. In one case neither solvent nor active ingredient (KBDO) were applied. From this, it appears that the paralysis was not related to the decontaminant applied.

To be scored as paralysed, an animal had to survive the toxic insult either to the end of the working day or, if the paralysis was not evident that day, to the following morning. In both cases, there was ample time to observe and record a paralytic result. All paralysed animals had been in convulsion, prostration or flaccid paralysis and were recovering. Recovering, in the case of paralysed subjects, was defined as an animal conscious, responsive but unable to move various portions of the body. If food and water were placed within reach, many of the

paralysed subjects would eat, drink and continue adjusting to the paralysis. In a few, more severe cases, the animals were breathing, sternally recumbent, unresponsive to external stimuli and would neither eat nor drink. In these cases, respiratory function, heart beat and reflexes could be established but mental awareness appeared absent.

The paralytic responses varied in the area and extent of the body affected (Table 1, Fig. 1). The largest group (37%) were paralysed below the neck *ie.* a limbs paralysed. The second largest group had both hind legs paralysed. Smaller numbers had only one limb affected. Affected limbs were stiff, rigid and immobile (Fig. 1). Paralysis of just paws or lower limbs was not observed. When the paralysis was first observed, some animals were allowed to continue for the 24 day observation period (the animals in Fig. 1 were of these) in the hope that remission might occur. As there was no improvement in these animals, all subsequent cases of paralysis were terminated once the condition was recognized. The incidence of paralysis in the different body regions (Table 2) did not follow any pattern but appeared random. A *Chi square* test of the incidence table indicated that the frequencies were similar ($P < 0.05$).

In the paralysed animals, the common histological finding was single or multi-focal damage in some area of cerebral cortex. The Veterinary Pathologist described the changes as "encephalomalacia". The pathologist wrote of one paralysed guinea pig "...in all grey matter of the brain there are many darkly basophilic, shrunken neurons without any detectable glial reaction. In addition, there are extensive malacic lesions in the cerebral cortex, in which many of the neurons are shrunken, with strongly acidophilic cytoplasm and pyknotic nuclei. Severe gliosis, both focal and diffuse, and perivascular lymphocytic cuffing, accompany these changes. In severely affected areas there is vacuolation of the neutrophil of the granular layer and the overlying meninges are infiltrated with lymphocytes. The pyriforme lobe and the adjacent gyri are most severely affected and the lesion is bilaterally symmetrical." In summary: - "Definite lesions of cerebral cortical malacia were found in guinea pig #7. This is a definite premortem lesion ...very similar, both in type and distribution, to lesions described in rats...[14]. Diagnosis - poliomalacia of cerebral cortex with gliosis and perivascular cuffing." For another pair of brains: - background changes are "...shrunken hyperchromatic neurons are found in all areas of the grey matter: some meningeal blood vessels contain increased numbers of neutrophils." In addition to background changes "...contracted, basophilic neurons are particularly common in the cerebral cortex, tending to have laminar distribution in the middle of the granular layer.....these changes (shrunken, hyperchromatic neurons) are very marked in the cerebral cortex at the midgranular layer....Diagnosis - laminar poliomalacia". For two other cases: - in addition to the background changes "...there is a single focus of astrogliosis located in the corpora quadridemia..." and "...there are scattered foci of vacuolation in the brainstem and cerebellar white matter ... Diagnoses -Focal gliosis and vacuolation of the brain."



Figure 1. Four views of two partially paralysed guinea pigs. In top left and right, the paralysis affects the left hind leg only. In the bottom views, the right hind leg is affected. Note that the opposite leg is unaffected and flexes.

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Table 1. Incidence of clinical signs in guinea pigs poisoned with GD.

Treatment	Number of guinea pigs	Per cent in populations	Statistics for dose of GD (LD ₅₀)			
			Mean	St. dev.	Min	Max
Total numbers	1277	100	8.4	±4.3	0.5	24
Dead	589	46	8.7	±4.1	2.0	24
Alive	688	54	8.1	±4.4	0.5	24
Animals alive						
Unaffected	340	49	7.1	±4.0	0.5	24
With toxicosis	348	51	9.1	±4.6	0.5	24
Affected survivors	348					
Ptyalism	332	95				
Tremors	108	31				
Convulsions	42	12				
Prostrate (flaccid)	99	28				
White tears	45	13				
Paralysis	84	24	8.8	±4.0	2	24
Where paralysed	84					
All quarters	31	37				
One side right ^a	2	2				
One side left ^b	1	1				
Front both	7	8				
Left only	4	6				
Right only	4	7				
Hind both	19	23				
Left only	2	2				
Right only	6	8				
Reflexes	8	9				
Decontaminant applied to paralysed guinea pigs						
Total paralysed	84	100				
Soap and water	1	1.2				
PEG only	14	17				
PEG KBDO	10	12				
MPEG only	11	13				
MPEG KBDO ^a	48	57				

^a RSDL®

Table 2. Incidence of clinical signs in guinea pigs poisoned with VX.

Treatment	Number of guinea pigs	Per cent in populations	Statistics for dose (LD ₅₀)			
			Mean	St. dev.	Min	Max
Total numbers	108	100	23.7	±8.1	4	36
Dead	69	64	23.5	±9.0	4	36
Alive	39	36	24.1	±6.2	16	36
Animals alive						
Unaffected	13	33	20.6	±4.9	16	32
With toxicosis	26	67	25.9	±6.0	16	36
Affected survivors	26					
Ptyalism	26	100				
Tremors	6	23				
Convulsions	4	15				
Prostrate (flaccid)	4	15				
White tears	0	0				
Paralysis	4	15				
Mortalities	69					
Ptyalism/tremors	69	100				
Convulsions/prostrate	69	100				
White tears	30	43				

All four paralysed guinea pigs were decontaminated with RSDL®

Effects of VX

In all, 108 guinea pigs were exposed to VX in these studies (Table 2). All were decontaminated with RSDL® after about one min exposure (55 to 60 sec according to the immediate experimental protocol applied). Of the 108, 36 per cent survived the VX challenge. All of the mortalities were the result of nerve agent poisoning and generally showed the classic signs of the organophosphate toxicosis. Of the survivors, 67 per cent showed clinical signs of VX toxicity and survived the insult. Four animals showed paralysis. The incidence of paralysis was 15 per cent in animals that survived and showed clinical signs. Of the total number affected, including mortalities, the incidence was four per cent.

The observed sequence of clinical signs was similar to that seen with GD albeit that the onset of signs was delayed - up to 2 hr - and the times-to-death were proportionally longer. Among the affected survivors, ptyalism was always observed and tremors were occasionally seen. All animals died that convulsed or became prostrate.

Again, because of the structure of the experiments, there was no relationship between dose of VX and the clinical signs observed (Table 2).

Discussion

To the authors' knowledge, this is the first formal report of partial paralysis as part of the immediate sequelae to organophosphate poisoning. The classical sequence of clinical signs; - ptialism, tremors, convulsions, flaccid paralysis, apnea and death - has been well documented in several texts [1, 2, 3, 4]. There is no mention in these texts of paralysis in the survivors of acute poisoning. One early paper [14], refers to paralysis occurring 7 - 14 days following exposure to organophosphates as an occasional outcome of "delayed neurotoxicity". It should be noted that the paralysed animals described in this report were all recovering from near death experiences. Paralysis was not observed in any less challenged animals. As in most animal studies, only readily visible clinical signs were recorded. No attempts were made to define either behavioural or psychic damage in the subjects.

Necrotic damage in the brain following organophosphate and nerve agent poisoning has been recognized for a long time and studied in several species [8, 10, 11, 13]. Delayed degenerative and necrotizing changes in the central nervous system and spinal cord have been reported in cases of human organophosphate exposure [15, 17]. In separate studies, similar brain damage has been reported following both GD and GB challenges in rats [10, 11, 14, 15, 16]. In a study specifically intended to demonstrate the brain damage by GD in rats, no lesions were found in the brain stem, spinal cord or sciatic nerve [14], albeit that the number of animals examined was small. Other organophosphates also cause similar brain damage [15]. To the authors' knowledge, this is the first report of brain damage in guinea pigs following organophosphate poisoning.

In the rat studies with GD and GB [10, 11, 15, 16, 14], the design of the experiments would tend to obscure the paralysis issue. Fixed, sub-lethal doses were applied to small numbers of animals which were terminated at fixed intervals to pursue histological studies. In contrast, in the work reported here, large numbers of animals were involved and many near death results were recorded. There were no attempts at medical intervention and no therapies were applied. The amount of agent absorbed was uncontrolled. The amount was that which could be/was absorbed in the window between contamination and decontamination. In many cases, the dose was very close to lethal and it was among these near lethal responses that the paralysis was identified. Because brain damage has been identified with direct exposures to GD and GB without decontamination, it is very likely that the brain damage reported here is from the same cause - i.e. organophosphate poisoning. Some form of functional deficit can be expected as a result of such brain damage. The paralysis reported here would be a logical result.

In the exposures to GD and VX described here, paralysis was observed only in animals that had been *in extremis*; - either convulsions or flaccid paralysis. All had been prostrate from advanced poisoning. In some cases, the animals were thought to be dead and yet recovered (This was early in the experiments when the workers were less familiar with the toxicosis). As apnea and flaccid paralysis following tonic spasm were a regular occurrence, there is the potential for anoxic damage to the brain and spinal cord. However, previous studies [10, 11] have indicated that anoxia may not be involved. The CNS lesions apparently can be prevented by therapeutic intervention with anti-convulsant drugs [9, 12, 13, 14].

There was no apparent relationship between the cutaneous doses of nerve agents and the occurrence of any of the clinical signs or the paralysis. Because the skin decontamination was generally effective and the work was approaching the optimal configuration for the decontaminant, the amounts of agent applied to the animals steadily increased. Eventually, the total amounts of agent in the open on the backs of guinea pigs before decontamination were so large ($>70 \mu\text{L}$ per animal; 26 animals at a time) that the work was stopped for safety reasons.

The incidences of clinical signs recorded in Tables 1 and 2 are those actually observed and may not present the full picture for each animal. The experiments from which the data were derived were conducted over a period of several years with the main object being the development of an effective skin decontaminant for chemical warfare agents. Specifically, the experiments were designed to demonstrate the potency of the RSDL[®] for decontaminating skin [23, 24, 25, 26, 27]. On a given day, the experiments usually involved a number of animals simultaneously and the experimenters were occupied with the contamination and decontamination procedures. Some animals went rapidly from convulsions to death when others were being decontaminated and washed. The result was that the occurrences and sequences of the clinical signs were not always recorded. Also, the convulsions were not always violent. As the animals were restrained, mild convulsions may have been overlooked. Further, some animals were in the early stages of toxicosis at the end of the working day and the progression of clinical signs may have occurred after hours.

In human exposures to nerve agents, the casualties may range from very slight contacts to near fatal cases [30, 31, 32]. In military operational exposures, it is probable that there will be cases with and without pretreatments and with a range of therapeutic treatments. In the cases where treatments have been applied, there will be little or no control over the timing of the interventions relative to the appearance of clinical signs, to time/duration of exposure or to the time from exposure or treatment to triage. Under these conditions, the sequelae described in the guinea pigs in this study may well be duplicated in human subjects and some of the survivors may be paralysed. The paralysed patient represents a significant drain on medical facilities for both immediate and long term care. Therefore, until there is evidence to the contrary, paralysis should be added to the list of concerns to be addressed when assessments are made of the medical requirements for treating, handling, recovery support and long term care of casualties from nerve agent poisonings.

Conclusions

Guinea pigs, exposed to G and VX such that flaccid paralysis results, may survive but have a reasonable chance of being paralysed from focal necrotic damage in the brain. The damage appears to randomly affect various limbs and combinations thereof according to the site of neurological damage.

The overall incidence of paralysis from GD was 9 per cent of those challenged and affected and 24 per cent of those recovering from advanced poisoning. Following VX challenge, the incidence values were 15 and 4 per cent, respectively.

Because similar histologic sequelae have been reported in rats and humans 7 - 14 days after poisoning, there is a strong possibility that the paralytic events described may also occur in

poisoning in humans and provide a serious complication in the treatment of personnel exposed to GD.

Until information is available to the contrary, it must be assumed that paralysis may result from challenge with all of the nerve agents

Recommendation

That paralysis be added to the list of concerns to be addressed when assessments are to be made of the medical requirements for treating, handling, recovery support and long term care of casualties from nerve agent attacks.

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Since the discovery of the G-type nerve agents, there have been numerous studies of Central Nervous System (CNS) involvement in the etiology of organophosphate poisoning, notably associated with *status elipticus* and/or "electrographic seizures". Brain damage from these effects has been demonstrated as "neuronal necrosis" primarily in the cortex, thalamus and hippocampus. However, despite recurring references to CNS damage, to the author's knowledge there have been no reports of long term paralysis manifested within 24 hr of nerve agent exposure. Occasional paralysis following an asymptomatic time interval of 7-14 days has been reported. This report is intended to summarize the immediate, nerve agent induced paralytic events recorded in guinea pigs (as incidental observations) during development of the Canadian Reactive Skin Decontaminant Lotion (RSDL®). Because the experiments were designed to assess the decontamination procedures, there were no apparent relationships between the amounts of agent applied and the sequellae recorded.

In the experiments described, massive cutaneous doses of agent were applied to large numbers of guinea pigs (GD to 1277; VX to 108) followed by decontamination with the RSDL® or predecessor lotions and solvents. The mortalities from GD (589) and VX (69) exhibited the classic clinical signs of nerve agent poisoning, namely excessive salivation (ptyalism), tremors, fasciculations, convulsions, apnea and flaccid paralysis before death. Of the 39 survivors of VX challenges, four exhibited the paralysis described following upon an insult which produced convulsions and/or flaccid paralysis. All of these were decontaminated with RSDL®. Of the survivors of GD challenges (688), many (348) exhibited some or all of these clinical signs. In addition, 84 animals recovering from the convulsions and/or flaccid paralysis showed apparently random, permanent paralyses of various parts of the body. Histological examinations of the brain and spinal cord of paralysed animals indicated encephalomalacia with occasional scattered foci of vacuolation in the brainstem and cerebellar white matter.

Of the 84 guinea pigs paralysed after GD challenge, one was not decontaminated, 25 were decontaminated with solvents only, either polyethylene glycol (PEG) or polyethylene glycol monomethylether (MPEG), 10 were decontaminated with potassium 2,3-butanedione monoximate (KBDO) - PEG and the remaining 48 were decontaminated with KBDO - MPEG (some variant of RSDL®). Thus, it would appear that the decontaminants had little effect on the resulting paralysis.

From these results, paralysis should be added to the list of concerns to be addressed when assessments are to be made of the medical requirements for treating, handling, support and long term care of casualties from nerve agent attacks.

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Nerve agents
GD
VX
Paralysis
Pathology
Guinea pigs